

The results revealed during the study period (2001-2010), an average prevalence of Bovine Tuberculosis in cattle for mainland Portugal is 0.10%, for Beira Interior is 0.09% and 0.42% in Beira Interior Sul. For the average prevalence in farms was 0.29% in Portugal, 0.24% in *NUTS II: Beira Interior* and 2.37% in *NUTS III: Beira Interior South*.

The results will lead to support decision-making in relation to cattle and their handling, and hunting and repopulation of wild ungulates.

A spatial statistical analysis applied to counties with outbreaks of TB in cattle was detected clusters and correlations in two parishes (Castelo Branco and Rosmaninhal) and dispersion in two others (Monforte and Monfortinho). The spatial autocorrelation (Global and Local) sensed distances between 13 and 25 km as being those where the grouping is more intense, and regarded as Hot Spot areas of the parishes of Malpica do Tejo, Monforte, Rosmaninhal, Ladoeiro, Segura and Zebreira.

The dispersion or clustering of outbreaks may be related to the proximity of the farms, with the scattering properties, with the exchange of animals between farms of the same owner, buying and selling animals or with health problems (s) Producer (s).

Regarding the activity of hunting, the beats and mounts can lead to deers are "pushed" to more remote locations, which increase their dispersal, which increases the likelihood of spread of pathogens.

The proximity to Spain may have some relationship in the areas of influence of hot spots determined by spatial statistics, since hunting wild animals roam freely and with ease between the two states.

## P242

### TUBERCULOSIS IN THE CZECH REPUBLIC AT RISK GROUPS: CASUISTRY FROM THE CURRENT TIME

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Tuberculosis is still an important infectious disease, as old as humanity itself. In the Czech Republic there is an ongoing trend of declining incidence of TB, including TB in children. In November 2010 the area-wide vaccination of children ended and in 2012 it was switched to selective vaccination of groups at-risk. The Czech Republic lying in the heart of Europe is a crossroad for migrants and immigrants bearing risk of importing tuberculosis, including its MDR-TB form. The most important risk group are foreigners from countries with high TB incidence, often with MDR-TB, the treatment of which is time-consuming and costly. Other risk groups are homeless people,

squatters, unemployed people, alcoholics, but also people under stress and work strain. We present the following case reports of TB patients from high-risk groups from recent time period: 1) Unvaccinated child of mixed marriage, a Czech mother, a father from Africa, treated for TB process. Child inspected as contact with TB father. TB for child confirmed by X-ray and also by gastric lavage and BAL: BACTEC MGIT 960 and cultivation positive, MTD test probable. 2) A boy from China, without proof of vaccination, grandfather treated in China for lung TB. TB confirmed by X-ray and from sputum: cultivation, BACTEC MGIT 960 and QuantiFERON positive, MTD test probable. 3) An unemployed man from Ukraine. TB confirmed from sputum: microscopy, cultivation and MTD test positive. 4) Female from Kyrgyzstan. TB confirmed by X-ray, cultivation and MTD test positive. 5) An unemployed man, alcoholic, living in squat, infected his roommate (contact). Sputum of both men: microscopy, cultivation and MTD test positive. 6) A student with TB infected his younger brother (contact). They both live in a "normal" functioning family, students, since childhood racing sportsmen. Sputum from both: microscopy, cultivation, BACTEC MGIT 960 and MTD test positive. Conclusions: Early diagnosis of new cases, prompt investigation of contacts, proper treatment, supervision of patients, taking into account the patient's country of origin, and not forgetting selective vaccination of children from risk groups. This all helps to keep the favorable trend of TB incidence in the Czech Republic.

## P250

### CORRELATION BETWEEN STREPTOMYCIN INTERMEDIATE-LEVEL RESISTANCE AND GIDB MUTATION IN AN ENDEMIC MULTIDRUG-RESISTANT TUBERCULOSIS CLUSTER

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Development of streptomycin-resistance in *Mycobacterium tuberculosis* is usually associated with mutations in *rpsL* and *rrs* genes, although up to

50% of clinical streptomycin-resistant isolates may present no mutation in either of these genes. The situation in Lisbon Health Region is similar, although mutations in *rrs* gene are only rarely detected. In the present report we investigate the role of *gidB* gene mutations in streptomycin resistance.

We have analyzed 52 streptomycin-resistant and 30 streptomycin-susceptible *Mycobacterium tuberculosis* clinical isolates by sequencing and endonuclease analysis of the *gidB* and *rpsL* genes. All clinical isolates were genotyped by 12-*loci* MIRU-VNTR. Semiquantitative drug susceptibility testing was also performed to a select set of isolates to assess the resistance levels towards streptomycin.

The *gidB* gene of 18 streptomycin-resistant isolates was sequenced and four missense mutations were found: F12L (1/18), L16R (18/18), A80P (4/18) and S100F (18/18). The remaining isolates were screened by endonuclease analysis for mutations A80P in *gidB* and K43R in *rpsL* gene. Overall, mutation A80P in *gidB* gene was found in 7 streptomycin-resistant isolates and 12 streptomycin-susceptible multidrug resistant isolates. Also noteworthy, comparison of the distribution of *gidB*, *rpsL* and *rrs* mutations revealed that *gidB* A80P mutation was only present in isolates without *rpsL* and *rrs* mutations. Moreover, this specific mutation was found among all isolates belonging to genetic cluster Q1.

Streptomycin quantitative drug susceptibility testing showed that isolates carrying the GidB A80P mutation were streptomycin intermediate-level resistant and that standard drug susceptibility testing yielded inconsistent results probably due to borderline resistance.

Bioinformatic analysis on the degree of conservation showed that the GidB A80P mutation is predicted to affect protein function.

We conclude that *gidB* mutations may explain the high number of streptomycin-resistant strains with no mutation in *rpsL* or *rrs*. These mutations might occasionally confer undetected streptomycin low-level resistance in regular drug susceptibility testing. Also, GidB A80P mutations may serve as surrogate markers for Q1 cluster isolates that are associated with multidrug/extensively drug-resistant tuberculosis.

## P257

### DANISH MYCOBACTERIUM TUBERCULOSIS OUTBREAK STRAIN IS SPREADING AMONG GREENLANDIC INUIT IN DENMARK AND GREENLAND

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Transmission of *Mycobacterium tuberculosis* continues at high rates among Greenlanders in Greenland and Denmark, with 203 and 450 notified cases per 10<sup>5</sup> populations year 2010, respectively. We can document, that the predominant Danish *M. tuberculosis* outbreak strain "C2/1112-15" has been transmitted to Greenlanders in Denmark, and subsequently to Greenland, where it is spreading at alarming rates, adding to the already heavy tuberculosis burden in this population group. It is now clear, that "C2/1112-15" is able to multiply in genetically very different populations. Thus, it might have the ability to spread even further keeping in mind the potential clinical consequences of strain diversity, e.g. the widely spread Beijing genotype. The introduction of the predominant C2/1112-15 *M. tuberculosis* strain into the Inuit community in the Arctic Circumpolar Region is an alarming tendency which deserves attention. We need to monitor whether this strain already have, or will, spread outside The Danish Kingdom.

## P258

### ACTIVE TRANSMISSION OF MYCOBACTERIUM TUBERCULOSIS (MT) CONTINUES AT SURPRISINGLY HIGH RATES IN DENMARK

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Active transmission of *Mycobacterium tuberculosis* (Mt) continues at surprisingly high rates in Denmark (DK). It is seen in specific high risk segments of the population with social problems such as homelessness, alcohol, and/or drug abuse. The patients are infected with the Danish "C"/1112-15 Mt outbreak strain, and the transmission is attributed to delayed diagnosis. The present situation demands increased focus on early tuberculosis diagnosis and control of transmission, and improved actions calls for prioritizing the area politically and economically.